

# Identification of Conserved Domains and Motifs for GRF Gene Family

Jafar Ahmadi, Nafiseh Noormohammadi, Sedigheh Fabriki Ourang

**Abstract**—GRF, Growth regulating factor, genes encode a novel class of plant-specific transcription factors. The GRF proteins play a role in the regulation of cell numbers in young and growing tissues and may act as transcription activations in growth and development of plants. Identification of GRF genes and their expression are important in plants to performance of the growth and development of various organs. In this study, to better understanding the structural and functional differences of GRFs family, 45 GRF proteins sequences in *A. thaliana*, *Z. mays*, *O. sativa*, *B. napus*, *B. rapa*, *H. vulgare* and *S. bicolor*, have been collected and analyzed through bioinformatics data mining. As a result, in secondary structure of GRFs, the number of alpha helices was more than beta sheets and in all of them QLQ domains were completely in the biggest alpha helix. In all GRFs, QLQ and WRC domains were completely protected except in AtGRF9. These proteins have no trans-membrane domain and due to have nuclear localization signals act in nuclear and they are component of unstable proteins in the test tube.

**Keywords**—Domain, Gene Family, GRF, Motif.

## I. INTRODUCTION

GRF Growth regulating factor, proteins play a role in the regulation of cell numbers in young and growing tissues and may act as transcription activations in growth and development of plants. The GRF family proteins contain the evolutionally conserved QLQ and WRC domains in their N-terminal region [1], [2]. The QLQ domain of GRF proteins is characterized by the conserved Gln-Leu-Gln residues, which has Phe in place of Leo [1]. The QLQ domain of GRFs shows similarity to the N-terminal region of the yeast SWI2/ SNF2 protein, another component of the SWI/SNF chromatin-remodeling complex in yeast. It suggests that QLQ domain may be involved in protein-protein interaction [3]. Another feature of QLQ domain is the absolute conservation of bulky aromatic/hydrophobic and acidic amino acid residues as Phe, Trp, Tyr, Leu, Glu or their equivalents in terms of chemical and radial properties, The Pro residue is also absolutely conserved [1]. This finding indicates that this amino acid residue is critical for the function of QLQ domain, probably or protein-protein interaction [2]. The WRC domain is also protected by the conserved residue (Trp, Arg, Cys) and contains two distinctive structural feature, namely many basic

Jafar Ahmadi is Associate Professor of Imam Khomeini International University, Qazvin, Iran (Corresponding author Address: Department of Plant Breeding, Engineering Faculty, Imam Khomeini International University, Qazvin, Iran, 34149-16818 (Tel: +982833901139; Fax: +982833780073; e-mail: njahmadi910@yahoo.com).

Nafiseh Noormohammadi was M.Sc. Student of Imam Khomeini International University, Qazvin, Iran.

Sedigheh Fabriki Ourang is Assistant Professor of Imam Khomeini International University, Qazvin, Iran (e-mail: s.ourang910@gmail.cm).

amino acids (Arg and Lys) and the conserved spacing of three Cys and one His residues, the putative zinc finger (C3H) motif. The basic amino acids are highly conserved in all the GRF horologes in other seed plants. The WRC domain also contains a functional nuclear localization signal [1]. It has been suggested that WRC domain functions in DNA binding [1], [2]. In the GRFs has been confirmed another conserved motif like GGPL, TQL, FFD, that presence of this motifs are very effective in the GRF proteins function in plant specific tissues and organs. These motifs are different in terms of level of protection, existence and probably this is one of the reasons for difference of the amount and type of the GRF protein effects in different plants.

In this study, With regard to the critical role of transcription factors on important biological processes, it is important and useful to know the biochemical and structural properties of GRF proteins.

## II. MATERIAL AND METHODS

### A. The Collection of Protein Sequences

45 GRF protein sequences in *Arabidopsis thaliana* (9 proteins), *Zea mays* (14 proteins), *Oryza sativa* (12 proteins), *Brassica napus* (4 proteins), *Brassica rapa* (4 proteins), *Hordeum vulgare* (1 protein), *Sorghum bicolor* (1 protein) were identified and collected after searching in NCBI (<http://www.ncbi.nlm.nih.gov>).

### B. Prediction of Conserved Domains

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### C. Alignment and Phylogenetic Tree

Sequence alignments to find similar and conserved regions were created by Clustal W (<http://www.ch.embnet.org/software/ClustalW>) and Clustal X softwares. Phylogenetic tree was constructed by MEGA4.1 software using 1000 bootstrap replicates. Rate of similarity between proteins sequences was calculated by clustalO program (<http://www.uniprot.org/align>).

## III. RESULTS AND DISCUSSION

Alignments confirmed conservation of QLQ and WRC domains and zinc finger motif (C3H) completely in all sequences except AtGRF9 which has Phe, a conservative substitution of Leo, in place of Leo in QLQ domain (Fig. 1). As well as predicted protein motifs that were previously

identified, TQL, FFD, GGPL and C3H are identified as motifs that they are involved in structure and act of GRF protein sequences. In AtGRF proteins, GGPL and TQL that had previously been identified, we have also confirmed the existence of these motifs. According to Kim's report [1], TQL motif was already identified in AtGRF1, AtGRF2, AtGRF3, AtGRF4, but we identified this motif only in AtGRF1 and AtGRF2. We also identified FFD motif in AtGRF3 and AtGRF4. Also according to Zhang's report [4], GGPL and TQL motifs were already identified in ZmGRF proteins. Moreover, we identified GGPL motif in ZmGRF8 and ZmGRF13. we found that C3H motif, it was not completely protected so that in these proteins Tyr and Arg residue, respectively, in ZmGRF2 and ZmGRF4 have been replaced instead of His residue. Choi et al. [5] previously reported TQL motifs in OsGRF proteins. We also identified FFD and GGPL motifs in OsGRF proteins. All of BnGRF and BrGRF proteins were shared TQL and GGPL motifs. We also identified TQL and FFD motifs in HvGRF1 and SbGRF1.

There were seven subfamilies in phylogenetic tree (Fig. 2). Subfamily A was divided into two sub-subfamilies, which ZmGRF1, ZmGRF5, ZmGRF6, OsGRF3, OsGRF4 and

HvGRF1 were into A1 sub-subfamily. Since these proteins in addition to QLQ and WRC domains were shared TQL and FFD motifs in their sequences, for this reason they have high similarity and have been located in a same subfamily. Probably this sequences similarity has caused that these proteins have a similar function. ZmGRF12, ZmGRF14 and OsGRF5 were into A2 sub-subfamily. These proteins were already into a subfamily in Zhang's report [6]. Also as Zhang previously reported, in C and B subfamilies were often presented AtGRF, ZmGRF, OsGRF proteins [6]. All BnGRF and BrGRF proteins, AtGRF1 and AtGRF2 were into D subfamily. All of these proteins are shared in GGPL motif. Proteins in *B. rapa* and *B. napus* had high similarity together; therefore proteins in this subfamily have similar operations. ZmGRF2, OsGRF7 and OsGRF8 were in E subfamily. OsGRF6 was independently in F subfamily. In G subfamily were ZmGRF8, ZmGRF13 and OsGRF9 that they are shared in GGPL motif in addition to other conserved domains and motifs.

Q...L...Q

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AtGRF1 96 KSDVSPYLQ-----YCRNSG-YLGGMMNTSNMHGNLLTGVKGPFSLTQWAELEEQOALI
AtGRF2 121 KNSLSPFLHQIPPPSYFRSSGGYSGGMMNMSMQGN-FTGVKGFPTLTQWAELEEQOALI
AtGRF3 65 -----DSS-----SRFPKMGSSFFSWAQWQEELEEQOALI
AtGRF4 66 -----DSSNSSSSSRFLKMGNFPSWAQWQEELEEQOALI
AtGRF5 1 -----MMSLSGSSGRTIGRPPFTPTQWEELEEQOALI
AtGRF6 1 -----MATRIPFTESQWEELEEQOALV
AtGRF7 44 -----TPYAGNLLGCYYPYPTINAQLKLELROAM I
AtGRF8 105 YTSSHSGMFTPAGSGSAAVTVADPFFSLSSSGEMRRSMNEDAGAAAFSEAQWHELELROANI
AtGRF9 8 -----MEQEEVEEERMRNKWPWMKAAQLMEFRMOALV
    
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(A)

C WRC C H

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ZmGRF1 113 PVLGR-----KLDPEPGRCRRTDGKKWRCSEAAAPDSKYCERHMHGRNRSRKPVETQLAPQSQPPAAAAYSAAPPLAAAATAATN
ZmGRF2 120 PERSGS-----ADPEPGRCRRTDGKKWRCSEAAVGDQKYCERYIKRGCHRSRKHVEGRKATPITADPTMAVSCGSLLSHSAVAWQQ
ZmGRF3 78 CFCMG--FTR-KADBDPEPGRCRRTDGKKWRCSEAAVDPDSKYCEKHMHRGNRSRKPVEMSLATPAPAPAPAPAPATATATSSPSPSYHR
ZmGRF4 176 LDFGHN-----PEPGRCRRTDGKKWRCSEAAVDPDSKYCERHMHGRNRSRKPVETQLAPQSQPPAAAAYSAAPPLAAAATAATN
ZmGRF5 102 PVLGR-----KVDPEPGRCRRTDGKKWRCSEAAAPDSKYCERHMHGRNRSRKPVETQLAPQSQPPAAAAYSAAPPLAAAATAATN
ZmGRF6 107 PVLGR-----KVDPEPGRCRRTDGKKWRCSEAAAPDSKYCERHMHGRNRSRKPVETQLAPQSQPPAAAAYSAAPPLAAAATAATN
ZmGRF7 71 CYCAGAPFVGRKAAEDPEPGRCRRTDGKKWRCSEAAVDPDSKYCEKHMHRGNRSRKPVEVTSSPAAGAAAAYRPSAISTISPPRAADAP
ZmGRF8 150 GTYNAD-----SDPEPGRCRRTDGKKWRCSEAAVDPDSKYCERHMHGRNRSRKPVENQPKKTKKEVPAAG-SLPCAGPQSLKKA
ZmGRF9 75 CFCMG--FSRKPADPEPGRCRRTDGKKWRCSEAAVDPDSKYCEKHMHRGNRSRKPVEMSLATPAPP-----ASSAAITSTSPSPSYHR
ZmGRF10 172 LDFGHN-----PEPGRCRRTDGKKWRCSEAAVDPDSKYCERHMHGRNRSRKPVETQLAPQSQPPAAAAYSAAPPLAAAATAATN
ZmGRF11 71 CYCAGAPFVGRKAAEDPEPGRCRRTDGKKWRCSEAAVDPDSKYCEKHMHRGNRSRKPVEVTSSPAAGAAAAYRPSAISTISPPRAADAP
ZmGRF12 97 AYVGR-----KLDPEPGRCRRTDGKKWRCSEAAVDPDSKYCERHMHGRNRSRKPVESKTESSPPQLSTVVTITTTREAAAATPLES
ZmGRF13 153 GYANAD-----SDPEPGRCRRTDGKKWRCSEAAVDPDSKYCERHMHGRNRSRKPVENQPKKTKKEVPAAG-SLPCAGPQSLKKA
ZmGRF14 94 AYVGR-----KLDPEPGRCRRTDGKKWRCSEAAVDPDSKYCERHMHGRNRSRKPVESKTESSPPAHPSPPQLSTVVTITTTREAAAATPLES
    
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(B)

GGPL

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OsGRF6 355 LKLSREYSPIGLGFAANRDEIVNQGAEANWPMFRDSLGGPLGEVLTKNNMPEARNCLS--
OsGRF7 299 -----SSNGGNTRASWIPGSWEASLGGPLGEFFTNTSSASDDKGSRH
OsGRF9 315 -----HAPNMACMQEDSISSSWEMPQGGPLGELTNTSKNPPDSSIMKPEA
    
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(C)

TQL

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BnGRF2a1 322 NQEKPSGNHHNQSSWPEELKSDWTQLSMSIPVASSSPSSSTAQDKTALSPLRLDLPIQSQQ
BnGRF2a2 325 NQNKP-----EELKSDWTQLSMSIPVASSSPSSSTAQDKTALSPLRLDLPIQSQQ
BnGRF2b2 340 NQDKHN-----HSSWPEERKSDWTQLSMSIPVASSSPSSSSTHITGEDKTTLSPLRLSQE
BnGRF2b1 340 NQDKHN-----HSSWPEERKSDWTQLSMSIPVASSSPSSSSTHITGEDKTTLSPLRLSQE
    
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(D)



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